ORIGINAL ARTICLE: REPRODUCTIVE ENDOCRINOLOGY

Female cancer survivors exposed to alkylating-agent chemotherapy have unique reproductive hormone profiles

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Objective: To evaluate reproductive hormone patterns in women exposed to alkylating-agent chemotherapy.
Design: Prospective cohort.
Setting: University hospital.
Patient(s): Normally menstruating mid-reproductive-age women (20–35 years old) who had previously been exposed to alkylating-agent chemotherapy for cancer treatment were compared with two healthy control populations: similarly-aged women and late-reproductive-age women (43–50 years old).
Intervention(s): Subjects collected daily urine samples for one cycle.
Main Outcome Measure(s): Integrated urinary pregnanediol glucuronide (PDG) and estrone conjugate (E1c) and urinary excretion of gonadotropins (FSH and LH).
Result(s): Thirty-eight women (13 survivors, 11 same-age control subjects, 14 late-reproductive-age control subjects) provided 1,082 urine samples. Cycle length, luteal phase length, and evidence of luteal activity were similar among the groups. As expected, ovarian reserve was impaired in cancer survivors compared with same-age control subjects but similar between survivors and late-reproductive-age control subjects. In contrast, survivors had total and peak PDG levels that were similar to same-age control subjects and higher than those observed in late-reproductive-age control subjects. Survivors had higher E1c levels than both same-age and late-reproductive-age control subjects. There was no difference in urinary gonadotropins among the groups.
Conclusion(s): Women exposed to alkylating agents have a unique reproductive hormone milieu that is not solely explained by age or ovarian reserve. The urinary hormone profile observed in survivors appears more similar to same-age control subjects than to late-reproductive-age women with similar ovarian reserve, which may suggest that age plays a more important role than ovarian reserve in the follicular dynamics of survivors. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Progesterone excretion, urinary hormones, PDG, E1c, cancer survivors

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Each year in the United States, more than 100,000 women and girls under the age of 45 years are diagnosed with cancer (1). Of these, ~12,400 are younger than 20 years of age at the time of diagnosis (2). Cancer therapies have improved substantially in recent years, leading to dramatic increases in survival. Currently, there are estimated to be more than 10 million adult cancer survivors in the United States. Among U.S. adults in their thirties, one in a thousand is a childhood cancer survivor (3, 4). As survival improves, there is increasing emphasis on optimizing health and quality of life among survivors.

Many cancer therapies are gonadotoxic, particularly alkylating-agent chemotherapy and pelvic radiation (5, 6). These treatments result in acute destruction of ovarian follicles, which results in an overall depletion of the ovarian follicular pool. Indeed, multiple studies have shown that cancer survivors have decreased measures of ovarian reserve compared

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with unexposed women of similar age (7). Depletion of the follicular pool may manifest clinically as menstrual cycle irregularity, subfertility, and premature menopause. Even among those who resume regular menses after treatment, the risk of infertility and early menopause is increased (6, 8).

Accelerated reproductive aging in mid-reproductive-aged cancer survivors parallels observations during natural reproductive aging in late-reproductive-age women (9). In the late reproductive years, follicular secretion of antimüllerian hormone (AMH) and inhibin B declines, resulting in decreased pituitary suppression and a subsequent rise in FSH (10–16). Ovarian antral follicle count also declines (17, 18). Furthermore, urinary hormone assessment reveals that compared with mid-reproductive-age women, women in the late reproductive years are more likely to demonstrate luteal phase dysfunction and have elevated levels of E_2 metabolites and gonadotropins (19–21). Thus, in addition to impairment in oocyte number, studies suggest that among late-reproductive-age women, the quality of the ovarian follicles declines over time, even in women with regular menses.

We know that chemotherapy causes decreases in oocyte number similarly to natural reproductive aging. In contrast, we do not know if the quality of the ovarian follicle is impaired. Indeed, little is known about how cancer therapy influences folliculogenesis and if ovulatory quality is compromised. The aim of the present study was to evaluate ovulatory function in cancer survivors compared with naturally aging women in their middle and late reproductive years.

METHODS

The Institutional Review Board at the University of Pennsylvania approved this prospective cohort study. We enrolled three groups of women: cancer survivors, similar-age control subjects, and late-reproductive-age control subjects. Survivors were eligible for enrollment if they were 20–35 years of age, previously exposed to alkylating-agent chemotherapy, currently healthy, and ≥ 1 year past the completion of cancer treatment. Healthy women 20–35 years of age with no history of infertility and no exposure to chemotherapy were eligible for enrollment as same-age control subjects. Healthy women 43–50 years of age with no history of infertility and no exposure to chemotherapy were recruited as late-reproductive-age control subjects. Subjects in all groups were required to have regular menstrual cycles every 21–35 days and to have a uterus and both ovaries.

Exclusion criteria included pregnancy or lactation within the previous 3 months, use of hormonal contraception or replacement within the previous 3 months, body mass index (BMI) >30 kg/m^2, and excessive exercise (defined as >1 h/d). In addition, subjects were excluded if they had any medical condition other than cancer associated with premature ovarian failure (i.e., Turner syndrome or fragile X premutation) or ovulatory dysfunction (i.e., thyroid disease, congenital adrenal hyperplasia, Cushing syndrome, hyperprolactinemia, or polycystic ovary syndrome). Subjects were identified from existing prospective cohort studies at the University of Pennsylvania and from the Cancer Survivorship Program at the Children's Hospital of Philadelphia, Abramson Cancer Center, and community referrals.

Study visits were scheduled in the early follicular phase and included a questionnaire to assess demographic information, transvaginal ultrasound for measurement of antral follicle count, and blood sample collection for measurement of FSH, E_2, and AMH. The subjects were then asked to collect a urine sample daily starting on the 1st day of the menstrual cycle for one menstrual cycle. The sample was collected from the first morning void, poured into prelabeled glycerol-containing tubes, and stored in the freezer in a prelabeled box. Samples were transported to the clinic in coolers. Samples were then thawed quickly with the use of hot water, centrifuged, aliquotted into 1–1.25 mL samples, and refrozen at -80°C. Frozen samples were then shipped to the University of Michigan CLASS Laboratory for urinary hormone analysis. The subjects were asked to keep a diary in which they recorded the collection time, spotting or bleeding, missing collection days, and any problems with the collection.

The following analytes were measured in the urine for each day of the cycle: FSH, LH, pregnanediol glucuronide (PDG), which is a metabolite of circulating progesterone, estrone conjugate (E1c), which is a metabolite of circulating estrogens, and creatinine. Urinary FSH and LH were measured by means of two-site chemiluminescent immunoassay with the use of two monoclonal antibodies. The sensitivity for the FSH assay was 1.05 mIU/mL, range 1.05–244 mIU/mL, and inter- and intra-assay variabilities 10.9% and 3.9%, respectively. For the LH assay, the sensitivity was 1.0 mIU/mL, the range 1.0–587 mIU/mL, and the inter- and intra-assay variabilities 10.7% and 4.8%, respectively. Creatinine was measured by means of a spectrophotometric assay with a sensitivity of 0.05 mg/mL, range of 0.05–1.4 mg/mL, inter-assay variability of 11.4%, and intra-assay variability of 4.3%. PDG and E1c were measured by means of competitive immunoassay with the use of direct chemoiluminometric technology. For the PDG assay, the sensitivity was 25 ng/mL, range 0.005–25 μg/mL, inter-assay variability 12.3%, and intra-assay variability 7.7%. The E1c assay had a sensitivity of 5 ng/mL, range of 5.1–408 ng/mL, inter-assay variability of 11.0%, and intra-assay variability of 7.8%. Urinary FSH, LH, PDG, and E1c were normalized to creatinine and integrated over one cycle to generate total cycle values. Peak values were also examined. Follicular and luteal integrated urinary hormone levels were assessed based on day of luteal transition, which was determined based on the LH peak. Early follicular phase serum samples were also obtained for the measurement of FSH, E_2, and AMH (Gen II assay) by the University of Pennsylvania Clinical Translational Research Center. Serum samples were analyzed with the use of ELISA kits for AMH (Gen II assay; Diagnostic Systems) and IRMA Coat-A-Count kits for FSH (Siemens) and E_2 (Diagnostic Products Corp.). The range of the AMH assay was 0.050–10.0 ng/mL, with a sensitivity 0.025 ng/mL, inter-assay variability $<8\%$, and intra-assay variability of 5%. The range of the FSH assay was 1.0–100 mIU/mL, with a sensitivity of 0.25 mIU/mL, inter-assay variability $<8\%$, and intra-assay variability $<4\%$. The range of the E_2 assay was 20–3,600 pg/mL, with a sensitivity of 7 pg/mL and